

662260" SH947260

FIG. 1
Prior Art

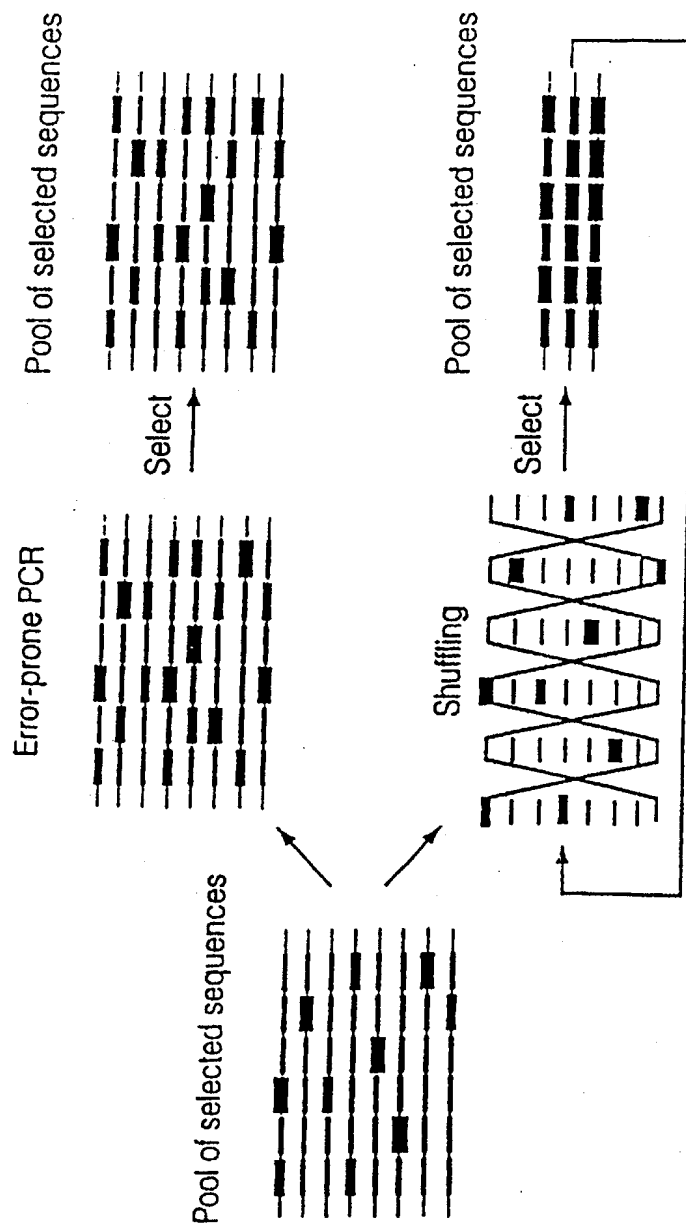


FIG. 2

Sexual PCR

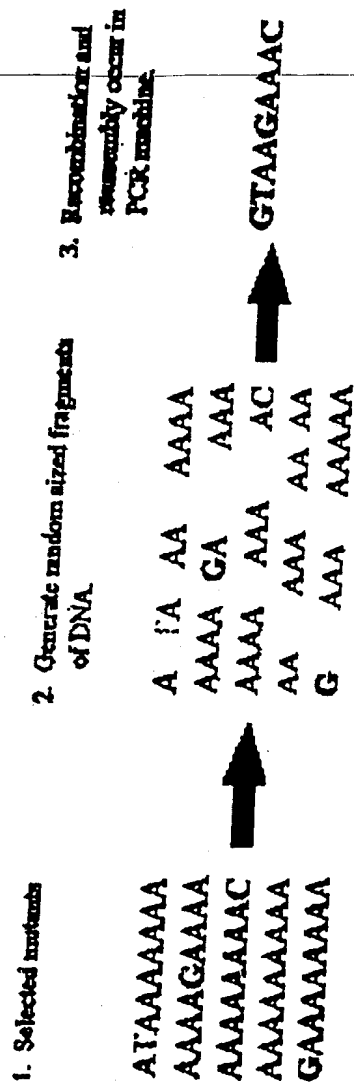


FIG. 3

DNA adducts for Sexual PCR

1. Random primers are used to amplify templates pretreated with DNA adducts.
2. Adducts cause premature termination of extension by blocking the polymerase.
3. Random size fragments are created by random priming and premature termination, not by digestion.
4. DNA fragments are ready for Sexual PCR.

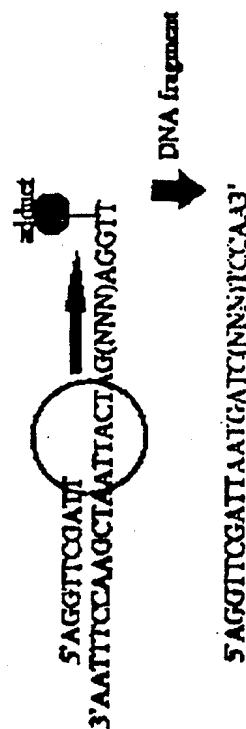
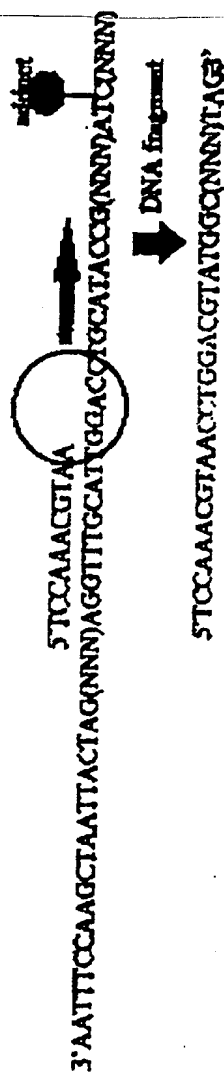


FIG. 4

DNA AdductsAnd UV
light

Aristolochic acid 1
Aristolochic acid 2
2-Amino-3-methylimidazo(4,5-f)quinoline
2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine
2-bromosacrolein (ZBA)
7-bromomethylbenzo(a)anthracene
benzo(a)pyrene
benzo(a)pyrene diepoxide
Mitomycin C
camptothecin
(+)-CC-1065 (from *Streptomyces zelensis*)
N-hydroxy-4'-fluoro-4-acetylaminobiphenyl
trivalent chromium
aromatic amines
platinum(II)
UV

FL 6.5

Creating DNA adducts using U.V. light

1. Irradiate pool of template DNA with U.V. light.



5' AGATTAAAGGAGTCGTAAGGATT3'
5' AGATTAAAGGAGTCGTAAGGATT3'
5' AGATTAAAGGAGTCGTAAGGATT3'

2. Cross links in the DNA will be introduced by the U.V. These crosslinks will stop Taq Polymerase extension.

5' AGATTAAAGGAGTCGTAAGGATT3'

5' AGATTAAAGGAGTCGTAAGGATT3'

5' AGATTAAAGGAGTCGTAAGGATT3'

3. Use random primers on cross linked DNA and extend with Taq Polymerase.

5' AGATTAAAGGAGTCGTAAGGATT3' ← 3'AGGCAAT5'

5' AGATTAAAGGAGTCGTAAGGATT3' ← 3'CCTAA5'

5' AGATTAAAGGAGTCGTAAGGATT3' ← 3'CTCAG5'

4. Taq extensions are blocked by U.V. adducts. This creates random size fragments ready for gene shuffling.

3'TCTAATTCCTCAGGCAT5'
3'AGGCATTCCTAA5'
3'AATTCCTCAG5'

FIG. 6

Reassembly of DNA fragments

Edd Lee

Fig 6A

lane 1- Isolated DNA fragments of mutant OCA₂ alkaline phosphatase gene,
length of ORF is 1.8kb
lane 2- 1kb ladder

1 2

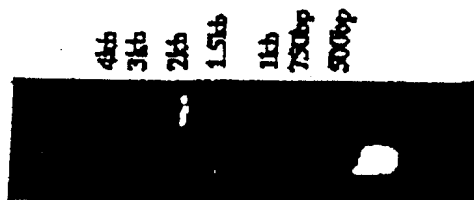


Fig 6B

lane 1- First round of reassembly, 1kb products have formed.
lane 2- Second round of reassembly
Predominant band at 1.8kb is the full alk phos ORF.
Reassembled product is ready for amplification, cloning and screening.
lane 3- 1kb ladder

1 2 3

